EXHIBIT 3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Cristobal Guillermo dos Remedios, et al.	**Examiner:**	Changhwa J. Cheu
Serial No:	09/778,259	**Art Unit:**	1641
Filed:	February 7, 2001	**Docket:**	13388
For:	BIOMOLECULAR TOXICITY ASSAY		

Confirmation No.: 4496

Commissioner for Patents
Alexandria, VA 22313-1450

DECLARATION OF DR. Cristobal Guillermo dos Remedios UNDER 37 C.F.R. §1.132

Sir:

I, Cristobal Guillermo dos Remedios, hereby declare as follows:

1. I am a co-inventor named in the above-identified application ("the '259 application").

2. I hold a Bachelor of Science (B.S.) degree in Science and Ph.D and D.Sc. degrees in Science, all from The University of Sydney. I have conducted research in the fields of biological macromolecules at The University of Sydney since 1974. I have authored 151 scientific publications in peer-reviewed journals including a publication relating to environmental pollution, I have edited 5 books and contributed 17 chapters in books, A true and correct copy of my curriculum vitae is attached hereto as **Exhibit A**.

3. I have reviewed the '259 application, and am familiar with the subject matter disclosed in the application. I understand that the claims of the application are directed to a method for detecting the presence of metal ions in an environmental sample.

4. I have also read the Office Action dated April 3, 2008, and I understand that Richardson et al. (*Environ Mutagenesis* 1981, 3:545-553) (hereinafter "Richardson") was cited in the Office Action as prior art against the claims in the '259 application. I have been asked to provide comments on this reference and its relevance, if any, to the method claimed in the '259 application, as well as on unexpected results that have been achieved by the '259 application relative to the art at the time when the '259 application was filed.

5. I have thoroughly reviewed the Richardson reference. This reference discloses measurement of the concentration of one metal ion in a laboratory-contrived solution. It is my opinion that those skilled in the art would understand the term "an environmental sample" to be a small amount of e.g. water, soil, air, gas that can be collected from an environment. It is also my opinion that those skilled in the art would not consider a solution prepared in a laboratory, which is employed by Richardson, to be an environmental sample. Further, in Richardson, each metal ion was analyzed independently, i.e., all the assays measured one metal ion at a time. There is no discussion in the reference on how to detect in a single assay, metal ions in an environmental sample, which typically contains more than one metal ions, in contrast to the method claimed in the '259 application.

6. Therefore, it is my opinion that the claimed method in the '259 application is different from the assay disclosed by Richardson, and further, the differences are substantial and significant. It is my opinion that it would not have been obvious to one skilled in the art how to arrive at the method claimed in the '259 application based on Richardson.

7. At the time the priority application of the '259 application was in 2000, the methods documented in the art for measuring toxicity of an environmental sample are generally live whole organism or cell-based assays. See, e.g., Barnes et al. (*JEB* 25(3): 270-275); Moulder (*Marine Biology* 59: 193-200, 1980); Skjak et al. (*J. Exp. Mar. Biol.* Ecol. 25: 37-50, 1976); Vranke et al. (*Marine Environmental Res.* 26: 161-1179, 1988); Kraak et al. (*Ecotoxicology & Environmental Safety* 25: 315-327, 1993); Rachlin et al. (*Arch. Environ. Contam. Toxicol.* 24: 16-20, 1993); Posthuman et al. (*Ecotoxicology & Environmental Safety* 38: 108-121, 1997); and Ince et al. (*Arch. Environ. Contam. Toxicol.* 36: 365-372, 1999). In

these prior art assays, the toxicity of an environmental sample is determined by assessing the effect of sample exposure on the viability and/or phenotype of the organism or cell.

8. The organism/cell-based assays, unlike molecular assays, were thought to best reflect the overall toxicity of an environmental sample because cellular defenses such as membrane barriers and cell receptors remained intact. By contrast, any molecule-based system, e.g., a nucleic acid based system of the '259 application, was widely considered incapable of providing a relevant or accurate measurement of toxicity for the reasons set forth below.

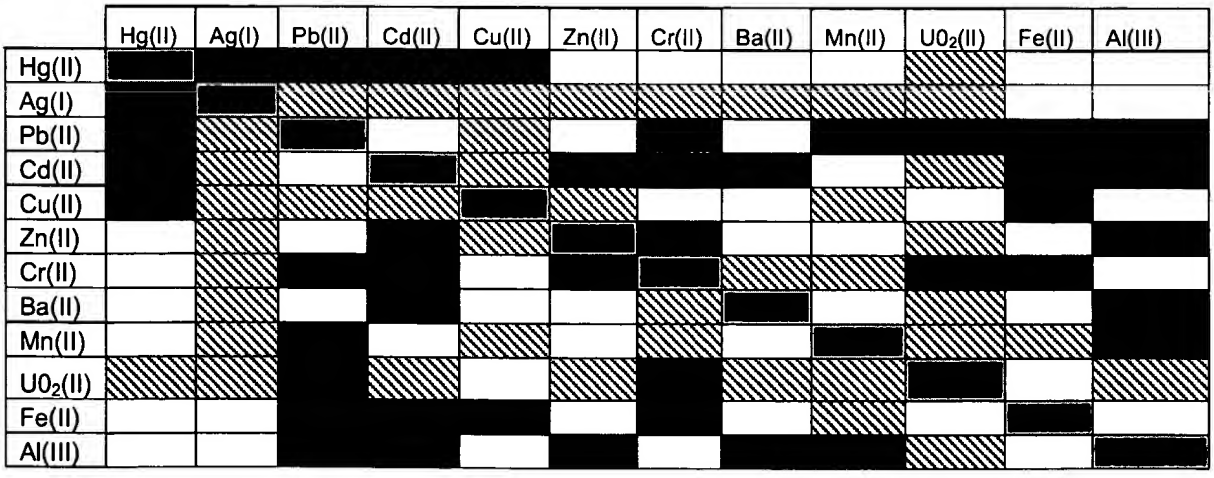
9. Multiple metals, which may be present in an environmental sample, do not necessarily merely have an simply additive toxic effect. The presence of one metal often has either a synergistic or an antagonistic effect on the activity of another metal in the sample, and *vice-versa*. This phenomenon affects the overall toxicity of the sample. For example, if Metal X and Metal Y each alone cause 1 arbitrary unit of toxicity, the additive effect would result in about 2 units, a synergistic effect would result in >2 units, and an antagonistic effect would result in <2 units.

10. The prevailing view in the art prior to the priority filing of the '259 application in 2000 was that synergistic or antagonistic effects of multiple metal toxicants could only be measured with live whole organism or cell-based assay systems. In several of the prior art references identified above, it is stated that the synergistic or antagonistic effects are a result of a complex reactions and interactions within the biological system which are used to measure toxicity. For example, one way antagonism is thought to occur is via competition for the binding sites on a cell surface. Molecular assay systems, e.g. "naked" nucleic acid based systems, were thought not to be useful for this purpose due to the lack of *inter alia*, cellular machinery (e.g. enzymes), cell/organelle membranes and associated adsorption sites, intracellular and trans-membrane import/export mechanisms and intracellular and membrane-bound receptor molecules. Up until the filing of the '259 application, a molecular assay system would have been expected by the person skilled in the art to only be capable of measuring an additive effect, possibly resulting in false positive or negative toxicity results. A nucleic acid

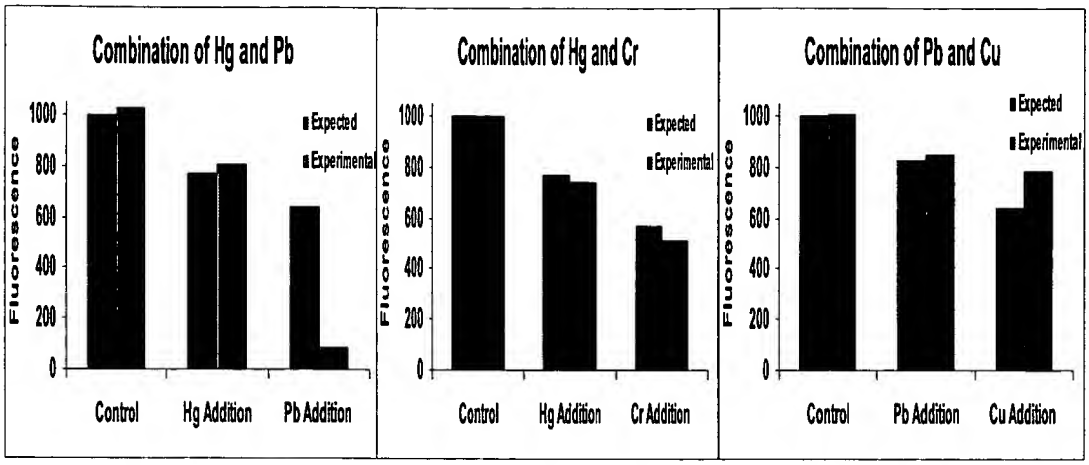
based assay, as disclosed and claimed in the '259 application, has now been demonstrated to be able to detect synergy and antagonism in mixtures of metals.

11. Table 1 below summarizes the types of interactions of heavy metal ions using the DNA-dye assay disclosed in the '259 application. Black boxes indicate a synergistic interaction (e.g. 1+1=2.5); Light down diagonal boxes indicate an antagonistic interaction (e.g. 1+1=1.5). White boxes indicate the expected effect, i.e. simply the additive effect of the two heavy metal ions (1+1=2). In these experiments, additional of a heavy metal ion dissociates the fluorescent dye resulting in a decrease in fluorescence intensity. The concentration of each of the heavy metal ions needed to produce a 25% fall in the fluorescence intensity was determined, then a second metal ion was added to the sample. An interaction was considered *additive* if the addition of the second metal ion fell within two standard deviations (SDs) of the expected fluorescence decrease (see Blue bar chart data). An interaction was considered *synergistic* if the addition of a second metal ion resulted in a fluorescence decrease greater than two SDs of the expected result (see green bar chart data). An interaction was considered *antagonistic* if the addition of a second metal ion resulted in a fluorescence decrease less than two SDs of the expected result (see red bar chart data).

Table 1



12. The following graphs show actual data from the DNA-dye experiments representing synergistic, additive and antagonistic interactions, respectively.



13. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment; or both, under Section 1001 of Title 18 of the United Stated Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: August 3, 2008